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MORPHOMETRIC AND IMMUNOHISTOCHEMICAL CHANGES IN LUNG TISSUE IN HYPERCHOLESTEROLEMIA: A REVIEW OF CONTEMPORARY EXPERIMENTAL STUDIES.

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Abstract

Hypercholesterolemia is traditionally regarded as a major systemic metabolic and cardiovascular risk factor, but experimental evidence increasingly demonstrates that disturbed cholesterol homeostasis also produces structural and functional abnormalities in the lung. The pulmonary consequences of hypercholesterolemia are particularly relevant because alveolar epithelial cells and alveolar macrophages actively synthesize, recycle, store, and export lipids, while pulmonary surfactant itself contains a substantial lipid component. This review summarizes contemporary experimental evidence concerning morphometric and immunohistochemical alterations in lung tissue under hypercholesterolemic conditions. Particular attention is given to alveolar septal thickness, mean linear intercept, airspace enlargement, lipid accumulation, collagen deposition, macrophage infiltration, oxidative stress, matrix metalloproteinases, TLR4/MyD88/NF-κB signaling, TGF-β/Smad activation, and the expression of CD68, α-SMA and other cellular markers. Experimental studies demonstrate that atherogenic diets and ApoE deficiency can induce pulmonary lipidosis, inflammatory infiltration, alveolar septal thickening and emphysematous remodeling. In ApoE-deficient mice receiving a Western-type diet, lung injury became more pronounced with increasing exposure duration, while TLR4 pathway inhibition attenuated these changes. Experimental disruption of ABCA1 or ABCG1

produces marked pulmonary cholesterol accumulation, foamy macrophages and surfactant abnormalities. In hypercholesterolemic rats, an eight-week diet containing 1% cholesterol was associated with oxidative stress, inflammatory cytokine elevation, collagen deposition and activation of TGF-β/Smad-dependent fibrotic signaling. Morphometric and immunohistochemical assessment therefore provides a more informative characterization of pulmonary injury than serum lipid measurements alone. The available evidence supports the concept that hypercholesterolemia can participate directly in pulmonary remodeling through the interaction of lipid accumulation, macrophage activation, oxidative stress, inflammation and extracellular matrix remodeling.

Keywords: hypercholesterolemia, lung morphology, morphometry, immunohistochemistry, alveolar septum, alveolar macrophages, CD68, α-SMA, cholesterol, pulmonary lipidosis, oxidative stress, pulmonary fibrosis, TGF-β, MMP, ApoE.

Introduction

Cholesterol is an essential component of pulmonary cell membranes and pulmonary surfactant, but its concentration and intracellular distribution must remain tightly regulated. The lung is not simply a passive recipient of circulating lipoproteins. Type II alveolar epithelial cells synthesize and recycle surfactant, whereas alveolar macrophages participate in the removal and processing of surfactant lipids. Consequently, disturbances in cholesterol uptake, intracellular storage or cholesterol efflux may rapidly influence the microscopic organization of the alveolar compartment [1]. The growing body of experimental evidence indicates that pulmonary lipid metabolism is closely connected with innate immunity, macrophage activation and tissue remodeling. Lipid-loaded macrophages, sometimes described as foamy macrophages, are increasingly recognized as a characteristic cellular phenotype in several pulmonary disorders rather than an incidental microscopic finding [2].

The importance of this relationship becomes clearer in experimental hypercholesterolemia. In C57BL/6J mice receiving an atherogenic high-cholesterol/high-fat diet containing 20% fat, 1.25% cholesterol and 0.5% sodium cholate for 12–16 weeks, pulmonary lipid accumulation progressively increased together with systemic hypercholesterolemia. Histological examination demonstrated accumulation of macrophages in alveolar spaces and lymphocytic infiltration around pulmonary vessels. At the molecular level, pulmonary TLR2 and TLR4 expression increased and nuclear translocation of NF-κB was activated [3]. Thus, the morphological lesion cannot be reduced to simple lipid deposition. Lipid accumulation is accompanied by activation of inflammatory signaling and cellular recruitment, which can subsequently alter alveolar architecture.

ApoE-deficient mice provide an even more pronounced experimental model because ApoE deficiency interferes with lipoprotein metabolism and markedly amplifies the response to an atherogenic diet. Ouyang et al. demonstrated that ApoE-deficient mice fed a Western-type diet developed lung lipidosis after 12 weeks, while pulmonary inflammation was time dependent. Leukocyte recruitment appeared at 4 weeks, increased alveolar septal thickness and mean linear intercept were observed at 12 weeks, and granulomatous changes became evident at 24 weeks [4]. TLR4, MyD88 and NF-κB were strongly upregulated at 12 weeks, and suppression of TLR4 signaling attenuated the pathological response. These observations are particularly important for morphometric studies because they demonstrate that structural remodeling of the lung evolves progressively rather than appearing as an immediate consequence of elevated serum cholesterol.

The relationship between lipid metabolism and pulmonary structure is also demonstrated by studies of cholesterol efflux pathways. ABCA1 and ABCG1 are central regulators of cellular cholesterol export. Experimental ABCA1 deficiency produces a striking pulmonary phenotype. Compared with wild-type mice, Abca1-deficient mice showed approximately 50% higher total lung cholesterol, a 17.5-fold

increase in cholesteryl ester, a 4.8-fold increase in cholesterol content of alveolar macrophages and marked abnormalities of surfactant homeostasis [5]. These findings establish a direct connection between defective intracellular cholesterol transport and pulmonary structural and physiological abnormalities. Similarly, ABCG1 deficiency produces progressive pulmonary lipidosis characterized by lipid deposition in alveolar macrophages and type II pneumocytes, accompanied by increased surfactant accumulation [6].

The objective of this review is therefore to analyze contemporary experimental evidence concerning morphometric and immunohistochemical changes in lung tissue during hypercholesterolemia, with particular attention to the cellular and molecular mechanisms responsible for pulmonary remodeling.

Materials and Methods

A narrative analytical review of experimental and translational literature was performed using combinations of the terms “hypercholesterolemia,” “high cholesterol diet,” “lung morphology,” “pulmonary lipidosis,” “alveolar macrophages,” “alveolar septal thickness,” “mean linear intercept,” “ApoE,” “ABCA1,” “ABCG1,” “TLR4,” “NF-κB,” “CD68,” “α-SMA,” “TGF-β,” “MMP,” “pulmonary fibrosis,” “oxidative stress,” “surfactant,” and related terms. Priority was given to experimental studies involving rodents, genetically modified animals, cell culture models and translational human investigations in which structural, morphometric, histological, immunohistochemical or molecular parameters of lung tissue were evaluated. Publications were selected from internationally indexed biomedical journals, with preference for studies providing quantitative measurements or clearly defined histological and molecular endpoints. More than 20 relevant publications were examined, and the 20 principal references were integrated into the manuscript in sequential citation order. Additional publications were used to broaden the interpretation of lipid metabolism, macrophage biology and pulmonary remodeling.

Results and Discussion

The available evidence demonstrates that pulmonary injury associated with hypercholesterolemia is characterized by several interconnected morphological phenotypes. The earliest changes may include lipid accumulation within alveolar macrophages and type II pneumocytes, followed by inflammatory infiltration, thickening of alveolar septa, alterations in airspace geometry and remodeling of the extracellular matrix. With more prolonged metabolic disturbance, collagen deposition and emphysematous enlargement of airspaces may become evident [4,7]. This progression is important because it indicates that hypercholesterolemia should not be evaluated solely through circulating total cholesterol or LDL-cholesterol. The pulmonary response is determined by how much cholesterol reaches the tissue, how efficiently it is exported, how macrophages process it and how the inflammatory microenvironment responds.

The alveolar macrophage represents one of the most sensitive cellular targets. Under physiological conditions, alveolar macrophages remove excess surfactant components and participate in local lipid recycling. When cholesterol efflux is impaired, macrophages accumulate neutral lipids and become morphologically foamy. In ABCG1-deficient mice, macrophage accumulation and inflammatory cell infiltration developed before severe pulmonary lipidosis, suggesting that immune activation may precede the maximal morphological accumulation of lipid [8]. Bone marrow transplantation experiments further supported the concept that macrophages are a major initiating cell population in ABCG1-associated pulmonary pathology. This observation changes the interpretation of CD68 immunohistochemistry. An increase in CD68-positive cells should not be considered merely as evidence of nonspecific inflammation; in the context of hypercholesterolemia it may reflect a metabolically altered macrophage population participating directly in lipid storage and inflammatory signaling.

ABCG1 deletion provides an especially clear example. In mice lacking ABCG1, extensive subpleural cellular accumulation, hypertrophy of type II pneumocytes, massive lipid deposition and increased surfactant levels developed with age. At three months, gross abnormalities were relatively limited, while marked changes became evident at older ages [6]. This age-dependent pattern suggests that compensatory mechanisms may initially maintain pulmonary lipid balance, but prolonged disruption eventually exceeds the adaptive capacity of the lung. From a morphometric perspective, this means that the duration of hypercholesterolemia is an essential experimental variable. Two animals with similar serum cholesterol levels may exhibit markedly different pulmonary morphology if the duration of exposure differs.

ABCA1 deficiency produces another distinct phenotype. Lung total cholesterol increased by approximately 50%, while cholesteryl ester increased 17.5-fold. Free cholesterol in the surfactant fraction increased by approximately 55%, and alveolar macrophage cholesterol increased approximately 4.8-fold [5]. These biochemical abnormalities were accompanied by alveolar proteinosis and respiratory distress. The morphological significance is considerable: the accumulation of cholesterol is not confined to the vascular compartment but extends into the alveolar epithelial–macrophage–surfactant system. Consequently, histological examination may reveal alveolar spaces containing proteinaceous material and lipid-rich macrophages rather than only vascular lesions.

The pulmonary surfactant system itself represents another important morphological-functional target. Surfactant is primarily synthesized by type II pneumocytes and contains phospholipids, cholesterol and specific surfactant proteins. Excessive or abnormal cholesterol incorporation can alter its biophysical properties. Experimental work in Wistar rats fed a high-cholesterol diet for 17–20 days found that serum hypercholesterolemia did not necessarily change surfactant cholesterol composition or predispose the animals to greater severity of several acute

lung injury models; nevertheless, isolated surfactant demonstrated impaired surface-tension-reducing capacity in a surfactant-depletion model [9]. This apparently negative finding is scientifically important. It demonstrates that hypercholesterolemia does not inevitably produce immediate structural injury, and that the pulmonary phenotype depends on exposure duration and the presence of additional injury stimuli. Therefore, absence of marked histological damage at an early time point should not be interpreted as evidence that pulmonary lipid metabolism remains unaffected.

The interaction between hypercholesterolemia and inflammatory signaling becomes especially clear in ApoE-deficient models. In ApoE−/− mice receiving a high-fat diet for 12 weeks, systemic TNF-α, IFN-γ, GM-CSF, RANTES, IL-1α, IL-2 and IL-12 were increased. Bronchoalveolar lavage fluid contained elevated TNF-α, IFN-γ and MIP-1α, while lung inflammation was dominated by monocytes and macrophages. Importantly, the inflammatory process correlated with collagen deposition and increased MMP-9 activity [7]. The finding links three major pathological events: inflammatory cell accumulation, extracellular matrix remodeling and structural distortion of the pulmonary interstitium.

A subsequent investigation of ApoE-deficient mice receiving a Western-type diet demonstrated a temporal sequence of pulmonary injury. At four weeks, leukocyte recruitment was evident; at 12 weeks, alveolar septa were thicker and mean linear intercept was increased; at 24 weeks, granuloma formation appeared [4]. This temporal sequence has direct implications for experimental morphometry. Alveolar septal thickness can provide an indicator of early-to-intermediate interstitial remodeling, whereas mean linear intercept reflects changes in the geometry of alveolar airspaces. An increase in both parameters may indicate that inflammation and interstitial thickening are occurring together with enlargement or distortion of alveolar spaces. These parameters therefore should ideally be assessed simultaneously rather than separately.

The role of TLR4 signaling is particularly convincing because molecular inhibition modified the morphological phenotype. ApoE−/− mice on a Western diet demonstrated marked increases in TLR4, MyD88 and NF-κB, while TLR4 knockdown reduced the associated pulmonary abnormalities [4]. In another model, an atherogenic diet containing 20% fat, 1.25% cholesterol and 0.5% sodium cholate induced pulmonary lipid accumulation in wild-type C57BL/6J mice over 12–16 weeks and increased TLR2/TLR4 expression and NF-κB nuclear activation [3]. The convergence of these two models suggests that cholesterol overload can activate innate immune pathways even in the absence of a genetically altered lipoprotein phenotype.

Oxidative stress adds another layer to the pathological process. High-cholesterol feeding in rats has been associated with increased pulmonary oxidative stress, inflammatory cytokines and fibrotic signaling. In a recent eight-week experiment, male Sprague-Dawley rats were divided into standard diet, standard diet plus 1% cholesterol, cholesterol plus atorvastatin and cholesterol plus ezetimibe groups. The hypercholesterolemic animals demonstrated pulmonary histological alterations and lipid accumulation, while treatment with atorvastatin or ezetimibe reduced oxidative stress, inflammatory cytokines and fibrotic mediators [10]. Lung tissue was assessed by hematoxylin-eosin and Oil Red O staining, and fibrotic changes were evaluated by Masson's trichrome staining. The study also demonstrated alterations in TGF-β/Smad signaling and multiple matrix metalloproteinases, including MMP-1, MMP-3, MMP-7, MMP-9, MMP-13 and MMP-19 [10]. This provides strong support for using combined histological and molecular evaluation rather than relying on one morphological parameter.

The immunohistochemical interpretation of TGF-β and α-SMA is particularly relevant when assessing pulmonary fibrosis. TGF-β is a central profibrotic mediator, while α-SMA is commonly used to identify activated myofibroblast-like cells. In the hypercholesterolemic rat model, TGF-β and TGF-β

receptor expression increased together with phosphorylated Smad2/3, while Masson's trichrome staining demonstrated greater collagen deposition [10]. The implication is that lipid-induced oxidative and inflammatory injury can progress toward activation of a classical fibrogenic pathway. Therefore, a combined assessment of TGF-β, p-Smad2/3 and α-SMA could provide a more mechanistically informative picture than α-SMA alone.

The extracellular matrix response is not uniform. ApoE−/− mice on a high-fat diet exhibited marked collagen deposition and increased MMP-9 activity [7]. In another hypercholesterolemic model, ApoE deficiency and a Western diet induced airspace enlargement together with increased MMP-9 activity and MMP-12 expression [11]. MMP-9 and MMP-12 are important because excessive proteolytic activity can disrupt the structural integrity of alveolar walls. Thus, hypercholesterolemia may theoretically produce two apparently opposite morphological outcomes: thickening of alveolar septa through inflammatory and fibrotic deposition, and enlargement of airspaces through matrix degradation. The final phenotype depends on the balance between inflammatory infiltration, collagen deposition, proteolysis and repair.

The connection between pulmonary lipid metabolism and smoking further reinforces this concept. Cigarette smoke rapidly induces lipid accumulation in pulmonary macrophages and promotes a foam-cell-like phenotype, with IL-1α release contributing to inflammation [12]. Smoke exposure also reduces macrophage cholesterol efflux through downregulation of ABCA1 and enhances TLR4/MyD88 signaling and MMP-9/MMP-13 expression [13]. Therefore, if hypercholesterolemia already increases lipid delivery to pulmonary macrophages, simultaneous suppression of cholesterol efflux by cigarette smoke could produce a more severe lipid-laden macrophage phenotype. This interaction is particularly relevant for experimental models in which hypercholesterolemia is combined with nicotine or cigarette smoke.

LXR signaling occupies an important position in this metabolic network. LXR regulates genes involved in cholesterol efflux, including ABCA1 and ABCG1. Pharmacological LXR activation during cigarette smoke exposure increased expression of lipid-export genes and cholesterol efflux capacity under some experimental conditions, but excessive activation during continued smoke exposure could also adversely influence alveolar macrophages and surfactant homeostasis [14]. This demonstrates that pulmonary lipid metabolism is not simply a matter of "more cholesterol equals more injury"; rather, the cellular context, transporter activity and inflammatory environment determine the outcome.

Recent evidence further emphasizes the physiological role of endogenous LXR signaling. Mice deficient in both LXRα and LXRβ developed pulmonary lipidosis, congestion, fibrosis and chronic inflammation. Type II pneumocytes showed abnormal lamellar bodies and reduced expression of genes involved in surfactant, cholesterol and phospholipid metabolism, while alveolar macrophages became foamy and showed abnormal lipid-metabolism gene expression [15]. These findings are highly relevant for immunohistochemical studies because changes in the expression of lipid-handling pathways can occur together with visible changes in cell morphology. The type II pneumocyte should therefore not be considered simply as a passive epithelial cell; its lipid metabolism is closely integrated with alveolar architecture.

The relationship between serum lipids and pulmonary structure is also supported by human data. In the Multi-Ethnic Study of Atherosclerosis, 6,700 adults were evaluated using CT-derived high-attenuation areas as a marker of subclinical interstitial lung abnormalities. Each standard-deviation decrease in HDL-C was associated with a 2.12% increase in high-attenuation areas. Among 1,216 participants with biomarker data, the same HDL-C decrement was associated with a 3.53% increase in serum MMP-7 and a 6.37% increase in surfactant protein A [16]. Although this is not an experimental animal study, it supports the biological

plausibility of the experimental observations and suggests that altered lipid metabolism may be associated with subclinical pulmonary remodeling in humans.

The significance of lipid-laden macrophages has become increasingly evident in recent literature. Experimental and clinical observations indicate that pulmonary lipid-laden macrophages are generated through an imbalance between lipid acquisition, storage, metabolism and export [2,17]. In pulmonary alveolar proteinosis and related conditions, alveolar macrophages become strongly enriched in lipids, while impaired surfactant clearance contributes to alveolar dysfunction. Recent lipidomic studies demonstrated that macrophage lipid burden decreases during successful treatment of pulmonary alveolar proteinosis and that macrophage lipid content correlates inversely with clinical improvement [18]. Although pulmonary alveolar proteinosis is not synonymous with hypercholesterolemia, these data provide a useful conceptual model: intracellular lipid accumulation is not simply a microscopic endpoint; it can reflect a functional failure of macrophage lipid processing.

The methodological value of immunohistochemistry becomes particularly apparent in this context. CD68 can be used to quantify macrophage accumulation, but the marker does not distinguish between resting and metabolically activated macrophages. Therefore, CD68 should ideally be interpreted together with lipid staining, inflammatory markers or cholesterol-handling proteins. ABCA1 and ABCG1 can provide information about cholesterol efflux capacity, whereas TLR4, NF-κB-related markers and inflammatory cytokines provide evidence of innate immune activation. TGF-β, phosphorylated Smad2/3 and α-SMA can characterize the transition toward fibrotic remodeling. SP-A or SP-C can help evaluate the integrity and functional state of the alveolar epithelial–surfactant system.

Another methodological issue is the choice of morphometric parameters. Alveolar septal thickness is particularly useful for detecting interstitial expansion, but its interpretation is influenced by lung inflation and fixation conditions. Mean linear

intercept is valuable for assessing airspace enlargement, but it should be measured using standardized stereological approaches. The numerical density of alveolar macrophages, volume density of collagen, thickness of respiratory portions and vascular density can complement these measurements. In a model where both inflammatory thickening and emphysematous enlargement occur, a single morphometric parameter can easily produce a misleading conclusion.

Additional evidence comes from experimental studies involving combined metabolic and environmental stressors. Physical inactivity, for example, can modify pulmonary inflammatory responses. In mice exposed to cigarette smoke for 24 weeks, extreme physical inactivity aggravated lung inflammation and emphysema compared with smoking alone [19]. Although this model did not reproduce isolated hypercholesterolemia, it is highly relevant to combined-risk-factor research because physical inactivity may coexist with dyslipidemia and can modify the pulmonary response to other insults. Therefore, experimental groups combining cholesterol loading with hypodynamia, nicotine or alcohol may reveal morphological changes that remain weak or absent when each factor is studied separately.

Alcohol provides another biologically plausible cofactor. Ethanol exposure can stimulate NADPH oxidase-dependent reactive oxygen species generation and increase MMP-12 expression in macrophages [20]. Because MMP-12 participates in extracellular matrix degradation, chronic alcohol exposure may theoretically intensify structural remodeling when combined with cholesterol-induced macrophage activation. This hypothesis is particularly relevant for experimental studies evaluating CD68-positive macrophage accumulation together with α-SMA, MMP-12 or collagen staining.

The therapeutic response of the experimental models also strengthens the interpretation that hypercholesterolemia contributes causally to pulmonary remodeling. In hypercholesterolemic rats, atorvastatin and ezetimibe not only reduced circulating lipid parameters but also reversed pulmonary histological

alterations, lipid accumulation, oxidative stress, inflammatory cytokine elevation and fibrotic signaling [10]. These findings are important because an intervention that modifies both serum lipid status and lung morphology provides stronger evidence for a mechanistic relationship than a simple correlation. Nevertheless, the available studies remain heterogeneous with respect to animal species, cholesterol dose, dietary composition, exposure duration and morphometric methodology.

The overall morphological picture can therefore be summarized as a continuum. At an early stage, increased cholesterol delivery and impaired efflux promote intracellular lipid accumulation in alveolar macrophages and type II pneumocytes. CD68-positive cells become more numerous, and Oil Red O staining reveals neutral lipid droplets. As the process continues, TLR2/TLR4 and NF-κB signaling increase, inflammatory cells accumulate and alveolar septa become thicker. Prolonged inflammation activates MMPs and profibrotic pathways, leading either to collagen accumulation and interstitial thickening or, in other models, to destruction of alveolar walls and enlargement of airspaces. The coexistence of these patterns explains why hypercholesterolemic lungs may show both fibrotic and emphysematous features depending on the experimental model.

For experimental morphology, the most informative design would therefore combine serum lipid analysis with lung morphometry, histological staining and immunohistochemistry. At minimum, total cholesterol, LDL-C, HDL-C and triglycerides should be measured together with alveolar septal thickness, mean linear intercept and collagen volume fraction. CD68 should be used for macrophage quantification, while α-SMA, TGF-β/p-Smad2/3 and selected MMPs can characterize remodeling. Oil Red O or equivalent lipid staining is particularly valuable because routine hematoxylin-eosin staining may underestimate the extent of lipid accumulation. This multimodal approach allows structural changes to be linked directly to lipid overload, inflammation and fibrosis rather than describing them as nonspecific “degenerative changes.”

Conclusion

Contemporary experimental evidence demonstrates that hypercholesterolemia is capable of producing measurable structural and cellular alterations in lung tissue. The dominant abnormalities include pulmonary lipid accumulation, foamy alveolar macrophages, type II pneumocyte alterations, alveolar septal thickening, inflammatory infiltration, collagen deposition, extracellular matrix remodeling and, in some models, enlargement of alveolar airspaces. The intensity of these changes depends strongly on the duration and type of hypercholesterolemic exposure and on the genetic background of the experimental animal.

Immunohistochemical and morphometric findings provide important mechanistic information. Increased CD68-positive macrophages reflect the cellular component of pulmonary lipid handling and inflammation, while altered ABCA1/ABCG1 expression indicates defective cholesterol efflux. TLR4/MyD88/NF-κB activation links lipid accumulation to innate inflammatory signaling. Increased TGF-β/p-Smad2/3 and α-SMA expression, together with collagen accumulation and altered MMP activity, indicates progression toward structural remodeling and fibrosis. The simultaneous assessment of these parameters therefore provides a much more complete picture than measurement of serum cholesterol alone.

The available evidence also suggests that additional risk factors such as cigarette smoke, nicotine, physical inactivity and alcohol may intensify pulmonary remodeling by converging on macrophage lipid accumulation, oxidative stress and extracellular matrix degradation. For this reason, experimental models of combined hypercholesterolemia and additional risk factors are particularly valuable for understanding the morphological progression of pulmonary injury. Further studies should focus on quantitative stereology, CD68-positive macrophage phenotyping, ABCA1/ABCG1 expression, α-SMA-positive myofibroblastic activation and TGF-β/Smad signaling in the same tissue samples. Such an integrated design could clarify

whether lipid accumulation is an early reversible phenomenon or a driver of persistent pulmonary structural remodeling.

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